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NONHETEROSEXUAL BEHAVIOR IN MOSQUITOES[1]

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Males of *Aedes aegypti* mosquitoes specifically use their antennae as phonoreceptors to pick up the buzzing sounds of the vibrating wings of flying females (Roth, 1948). Sound is the primary sexual cue given to the male. He must critically perceive sound to recognize the very presence of a female and then, still using sound, normally may locate and then choose a female with great precision and speed as she flies through the air (Jones, 1974). Males rapidly chase and capture females on the wing. Recognition, location and capturing of the female all depend on sound. Using his tarsi, the male quickly orients himself ventrally to the female and seizes her terminalium with his claspers. After terminalial contact, the aedeagus of the male may be inserted into the vagina and after this he may ejaculate into her bursa (Jones and Wheeler, 1965).

In his detailed study on the sexual activities of *Aedes aegypti*, Roth (1948) observed what he referred to as being "homosexual" behavior among the males, and he reported very high rates in the 2 counts he made on his strain. Male couples can be seen in colony cages in the laboratory under routine conditions.

It was the purpose of the present study to reexamine the sexual behavior of male mosquitoes relative to one another under various experimental conditions.

Materials and Methods

The Bangkok and U. S. Naval Medical strains of *Aedes* (*Stegomyia*) *aegypti* (Linnaeus) were mostly studied under free-flying conditions in a one cu ft cage with clear plastic sides in an insectary at 27° C and 70% relative humidity. All observations were made between 0830 and 1030 hours. The term "sexual encounter" includes a great variety of different acts: a distinct chase with or without capture, a midair capture without genital contact, a midair capture with genital contact, and capture with subsequent landing (with or without genital contact). Since it was not possible to observe individual couples in a large group for more than 10 to 15 sec, the same couple could have been recounted at least twice, especially if a couple were flying or if they landed and reflew as a couple. Ten percent sucrose was available except during the periods of observation and experiment.

The following terms and their definitions are critical. *Homosexuality* is well-defined as attraction towards members of the same sex (Dorland, 1974). The key word in this definition is attraction: the homosexual animal must perceive the other animal as being of the same sex and proceed towards it. For true homosexuality to occur, a specific choice of one sex must be made and the opposite sex avoided (White, 1963). *Heterosexuality* is defined here as attraction towards

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individuals of the opposite sex, with no attraction towards individuals of the same sex. *Bisexuality* is defined as equal attraction towards individuals of both sexes. *Nonheterosexual* behavior refers to mating activities between members of the same sex which are neither homosexual nor bisexual in character. *Copulation* will be defined as the insertion of a copulatory organ into an animal orifice.

Results

General flight activities of males

One day after emergence, adult males of *A. aegypti* either rest on the sides of a one cu ft cage or fly for relatively long periods of time. They normally fly quite rapidly in the confines of a one cu ft cage and make zigzagging flights as they move from side to side or fly up or down. They readily make sudden looping flights and can swiftly fly downwards for 10–20 cm without landing. Males fly very fast when they give chase to other mosquitoes. They appear to slow up as they first turn to follow another individual. Males in flight manuever with great speed and agility, and, in the majority of cases, make no attempts to capture each other, when they are of the same age. Males can fly very briefly "face to face." In a cage containing 100 males they often come within about 3 mm of each other and then dart away.

Description of nonheterosexual behavior

Nonheterosexual behavior was exhibited only among adult males and was never observed between females.

In a cage containing only males, single individuals can be seen suddenly chasing another male. Such chases often do not result in capture by the agressive male. Midair captures of one male by another were often seen where the couple very quickly broke off all contact just before they landed on the floor of the cage. Only very rarely would a male couple continue to fly together for longer than 5 sec. Many male couples were observed to fall to the floor of the cage and to be grossly misaligned relative to each other. Such couples usually parted after a brief struggle or after a variable period of mutual catatonia. Sometimes, however, one of the aggressive males would quickly thrust his terminalium in the general direction of the other, without making any terminalial contact with the passive partner. Aggressive males were generally located ventral to the passive male or else along one side. When a male couple happened to land so that the aggressive partner was on top of a captured male (which landed on his back), the top-mounted male made no terminalial thrusts, and generally quickly flew off. In the most clear-cut form of nonheterosexual behavior, the aggressive male first chases and quickly captures a passive male and the couple land on the floor with the aggressive partner ventral to and aligned with the passive male. Then, the aggressive male quickly seizes the claspers of the other male in such a way that very close genital contact occurs. Genital contact among such free males is always very brief and lasts about one sec or less. The aggressive male may make repeated efforts to make genital contact and the couple may struggle on the floor for a relatively long time, turning over and over. The couple will sometimes fly for

a short distance with the aggressor still attempting to grasp the other male's terminalium. In between these struggles, the couple may stand in mutual catatonia. Eventually, the struggles lead to separation of the pair.

It was frequently observed that the aggressive males usually did not have distended abdomens and that they would very commonly capture those older males which had their abdomens distended with sugar solution.

Sometimes in a cage of mixed sexes, a female would attract 2 or sometimes 3 males and after these had fallen to the floor, one of the males would usually copulate with the female, and, afterwards, this aggressive male might make a series of abdominal thrusts at the male who happened to be closest to him in the group. Sometimes 2 out of 3 males in association with one female would make abdominal thrusts at each other.

Levels of sexual activity in an all-male population

One hundred newly-emerged males were placed in a one cu ft cage and were observed over a period of nearly 12 days. Observations were usually made on undisturbed individuals for a 30 min period on each day they were studied. On the first six days, the animals were also deliberately disturbed, generally at one min intervals over another 30 min period. As shown in Table I, no nonheterosexual behavior was observed among 100 males during the first 24 to 36 hr under undisturbed cage conditions. After this time (from 48 to 276 hr), 10 to 20 nonheterosexual encounters were observed in a 30 min period. Deliberate shaking of the cage to disturb the males increased the number of males flying only for a brief period of time and did not specifically increase the level of nonheterosexuality in the cage.

TABLE I

Nonheterosexual encounters in an all-male population of Aedes aegypti.

Age of males	Number in cage	Male couples	
		Undisturbed cage 30 min	Disturbed cage 30 min
0–12 hr	100	0	0
3–15 hr	100	0	0
24–36 hr	100	0	2
48–60 hr	99	10	12
72–84 hr	99	20	11
96–108 hr	99	14	11
168–180 hr	99	17	—
192–204 hr	99	15	—
216–228 hr	96	20	—
240–252 hr	95	17	—
10 newly-emerged males added to cage		—	16
24–276 hr	104	19	—

Effect of isolating male couples on sexual activity

When the all-male population in the cage was 3 days old, those couples which were found together on the floor of the cage were quickly aspirated into another one cu ft cage. In all, 20 couples were so isolated. All 40 males flew very actively back and forth and often came very close together yet none of them made any attempts to chase or capture other males during an hour of careful study. This finding specifically and clearly demonstrates that the activity seen in the larger cage is not true homosexuality. When 40 virgin females were placed in the cage, there was a very great increase in sexual activity, and the females were quickly captured by the males and copulated with. Thirty min later, when the females were dissected, all of them had been inseminated and impregnated.

Experimentally increasing the nonheterosexual rate in a male population

The maximum level of nonheterosexual activity at any one moment in the all-male population under either undisturbed or disturbed conditions varied from 3 to 4 couples. When a tuning fork of 512 cycles per sec was struck within the cage, the males formed dense swirling masses around the fork, and there was a very marked increase in nonheterosexual captures during the short time the fork was actually vibrating. As many as 11 male couples were counted within 32 sec after striking the tuning fork. As many as 9 couples were seen on the floor of the cage at the same time. This was the maximum number of couples seen at any one time in this study. These male couples were nearly all very loosely attached only by one tarsus, and the insects stood side by side. Most couples made no attempts to establish genital contact and quickly broke up. Sometimes a group of 3 males was seen together.

When the males were 10 days old, the undisturbed nonheterosexual rate was 17 couples within a 30 min period. After this, 10 newly-emerged males were added to the cage and the number of male couples were counted over the next 30 min period. The nonheterosexual rate for this period was 16; that is, there was no increase in the rate. It was observed that many of the young males were indeed preferred to the old ones. It was also noted, however, that the older males frequently did not attempt to establish genital contact with them, even though they had been captured.

Since newly-emerged adult males are not only reluctant to fly at all but fly only for very short distances, it was of great interest to learn how it was possible for them to be captured at all by older males. It was soon observed that older aggressive males often landed very close to the newly-emerged males shortly after they had been introduced into the cage. The older males generally had one tarsus in contact with one of the legs of the young males. Thus, as soon as the young male flew, he would be immediately accessible to capture. Aggressive males also frequently land beside females in this way and more quickly capture them as well.

The young males were left in the cage overnight, and the next day a nonheterosexual rate was taken and estimated to be 19 couples in a 30 min period.

Ten 3-day old virgin females were then placed in the cage with 104 males. When heterosexual and nonheterosexual rates were counted over a 30 min period,

it was found that there were 71 heterosexual contacts and 142 nonheterosexual encounters within the first 30 min of placing the females in the cage. There was, thus, an enormous increase in general sexual activity. Although the nonheterosexual rate increased 8.6-fold, the maximum number of male couples on the floor at any one time was only 5. A number of male trios were also seen, far more than under any other experimental conditions. Some individual females were also picked up by 3 to 4 males at one time.

An attempt was made to compare the relative "attractiveness" of 5 recently-emerged young males with 5 3-day old females to a group of 10 3-day old males. A good comparison was not possible because most of the young males did not fly, whereas most of the females were very active fliers. First, 5 females were placed in a one cu ft cage and then 12-hr old males were introduced. None of the young males chased the flying females for a 30 min period. Only 2 of the 5 young males flew briefly when the cage was disturbed. A group of 10 3-day old males was then placed in the cage. Within 30 sec one male couple was seen with the aggressive male making repeated terminalial thrusts for a relatively long period of time. During the 30 min period of observation, this was the only male couple seen. During this same period, there were 63 heterosexual encounters.

Sexual activity in a heterosexual population

The final proof of a state of nonheterosexuality versus homosexuality would be to show that when there was an equal number of sexes of the same ages within a given population, there would be a very marked decrease in male couplings. As shown in Table II, when 50 males were placed with an equal number of females when they were less than one day old, heterosexual behavior was the predominant or only sexual activity seen over an 11 day period. Since the level of sexual

TABLE II

Extent of heterosexual and nonheterosexual activity in a population of Aedes aegypti *when the sex ratio is 1:1.*

Age of the mosquitoes	Number in cage	Number of encounters in 30 min	
		Heterosexual couples	Possible nonheterosexual couples*
0–18 hr	100	7	1
24–48 hr	100	74	1
48–66 hr	100	85	5
72–100 hr	99	85	3
96–124 hr	99	99	6
168–196 hr	98	81	1
192–220 hr	96	294	5
216–244 hr	90	224	1
240–268 hr	89	203	1

* Since the males were very similar in size to the females in this population, and since male couplings were very brief, it was not possible to make accurate counts. Most of the values given in this column were noted as being possible male couplings.

activity was very high under undisturbed conditions, no deliberate attempts were made to disturb the mosquitoes. None of the male couples showed any attempts to seize the other's terminalium.

Discussion

The word homosexuality is often used very loosely to refer to a great variety of undefined associations and ill-defined acts involving individuals of various species of the same sex. If the word homosexuality is defined as being a specific attraction towards members of the same sex (Dorland 1974), with avoidance of the opposite sex (White, 1963), then it is evident that male mosquitoes do not exhibit homosexuality. That is, when these animals are given a specific choice, they choose females and specifically avoid other males under heterosexual conditions. Even when the males are kept continuously in an all-male group, most of them specifically avoid each other. The level of male couplings in the all-male population used in this study was estimated as involving about 16% of the individuals over a 30 min period under undisturbed conditions in a one cu ft cage. One-half of these animals were aggressive males (that is, they captured other males which were their passive partners). This means that only about 8% of the population actually chase and capture other males. Since one aggressive male may have repeatedly captured one or more males, he could easily have been counted on more than one occasion during a 30 min period, so the 8% level of aggressive males is probably too high, by a factor of at least 2.

The estimated level of nonheterosexual behavior in an all-male population in the presence of newly-emerged males in a 30 min period under disturbed conditions is extraordinarily less than that recorded by Roth (1948) where he counted 98 male couplings within a 15 min period among only 22 males (4 young males with 18 old males). This averages about one sexual encounter every 9.2 sec! If each of the 4 young males were captured uniformly by older males (which is most unlikely), this would mean that each young male would be picked up about 25 times in a 15 min period.

In the present work, only 16 male couples were seen within a 30 min period among a group of 105 mosquitoes, where there were 9.5 times as many old males as young ones.

Roth (1948) believed that *A. aegypti* males would sometimes attempt to "copulate" with each other. Jones and Wheeler (1965) rubbed the terminalia of 2 restrained males together and observed that although one male would sometimes grasp the claspers of another with his own claspers, the aggressive male never erected his paraprocts or aedeagus, thus indicating that actual copulation between 2 males does not in fact occur. Observations made during the present studies support the observations of Jones and Wheeler (1965).

It is of considerable interest that the majority of aggressive males in nonheterosexual encounters seem to recognize they do not have a female partner before terminalial contact is made and quickly separate before landing occurs. Some more aggressive males capure another male and make terminalial thrusts and then depart without any terminalial contact beween the partners. Some aggressive males seize the terminalia of their partners a single time and at that

moment seem to recognize they do not have a female. A very few aggressive males make repeated terminalial thrusts and may repeatedly seize the claspers of another male before they recognize they are not in contact with a female.

In many animals the males do not recognize the sex of their partners until after the capture (Beach, 1938). The biological "philosophy" of a trial-and-error method is probably: It is better to grab and take a chance on having the right partner than no partner at all. Only the most aggressive males of *Aedes aegypti* seem to have this "philosophy"; most males avoid other males and most of them choose females with considerable accuracy. They do this by sound alone. The remarkable thing is that with this one sexual clue, the males make so few mistakes. Apparently, the males receive some additional cue from females prior to copulation, and this cue is missing from males.

It is concluded that a relatively low level of nonheterosexual activity occurs in most populations of *Aedes aegypti*. This phenomenon is the result of a momentary misperception of sound by aggressive males which mistake other males for females.

The most striking feature of nonheterosexual activity in *A. aegypti* is that most of the aggressive males which have captured another male make no attempts to orient themselves to the body of passive partner. This failure seems to be due primarily to the fact that most aggressor males do not land in a ventral position relative to their partners. If the aggressive males, however, land in a ventral position relative to their partner, then they often will make attempts at alignment and may thrust their abdomens in the direction of the other. In most cases, however, ventrally oriented aggressive males do not make genital contact. In a few cases, however, the aggressive partner not only attains a ventral position but is correctly aligned and actually establishes genital contact, and may do this more than once with the same male. In all such cases, however, the terminalial contacts were very brief (for less than one sec), strikingly more so than genital contacts made with free-flying females, where the unions vary from 1.2 to 84.6 sec (averages of 11 sec for 55 cases) (Jones, 1974). Males may be misaligned relative to captured females but they very often correct the misalignment and copulate.

Nonheterosexual activity differs in many ways from heterosexual behavior in *A. aegypti:* it begins later, involves relatively few individuals, tends to remain at about the same level over a period of days, is not generally increased by shaking the cage, rarely involves the formation of trios, and generally does not involve terminalial contact. If such contact occurs, it is significantly more brief than in heterosexual contacts.

It is proposed that nonheterosexuality in *A. aegypti* occurs when aggressive males become highly excited and are very close to other males which they momentarily perceive as being female.

Nonheterosexual behavior differs critically from homosexuality in that it does not involve a specific choice of the same sex. It differs from bisexuality in that the aggressive partner is not equally attracted to both sexes and generally selects the opposite sex. It seems probable that categorizations of "homosexuality" among many different animals will prove to be examples of nonheterosexual behavior.

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Summary

1. Adult males of *Aedes aegypti* usually avoid each other in flight but sometimes a few of them will chase and capture another flying male. Most of these aggressive males are misaligned and most make no attempt to align or grasp the terminalium of the passive partner. When terminalial clasping occurs, it is always very brief.

2. When 3-day old males are given a choice of contemporaneous or recently-emerged males, they choose the young ones. When given a choice between males and females, males almost invariably choose females.

3. The level of nonheterosexual activity in an all-male cage was measured over a 30 min period over an 11 day span and was first seen when the insects were 36 hr old. From 48 to 276 hr, the level of sexual activity in the cage was found to involve between 10 and 20% of the individuals, thus indicating that 5 to 10% of the animals are aggressive males.

4. All 20 male couples which were isolated from an all-male population flew, yet none of them were chased or captured in a 30 min period. When 40 females were placed in their cage, all the females were quickly chased and copulated with, and none of the males were chased.

5. When a tuning fork of 512 cycles was sounded in an all-male cage of 89 mosquitoes, many males formed dense seething swirls around the fork and as many as 11 couples were formed within the first 32 secs. Most of the individuals in the artifically-induced couplings stood side by side and no attempts were made to orient to or clasp the genitialia of the other individual.

6. Nonheterosexual activity differs from heterosexual behavior in that it appears later, involves relatively few individuals, occurs at a low level for a relatively long period, is not generally increased by shaking the cage, rarely involves trios, and generally does not involve terminalial contact. If such contact is made, it is significantly less than with females.

7. Nonheterosexuality in *A. aegypti* tends to occur when aggressive males become highly excited and are close to other males which they momentarily perceive as being female.

8. Nonheterosexual behavior differs from homosexuality in that it does not involve a specific choice of the same sex. The phenomenon differs from bisexuality in that the aggressive partner is not attracted equally to both sexes and is principally attracted by the opposite sex.

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